American Journal of Epidemiology
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Vol. 164, No. 11
DOI: 10.1093/aje/kwj301
Advance Access publication September 6, 2006

Invited Commentary

Invited Commentary: Disinfection By-Products and Pregnancy Loss—Lessons

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Received for publication June 19, 2006; accepted for publication June 21, 2006.

Abbreviations: BDCM, bromodichloromethane; DBP, disinfection by-product; THM, trihalomethane.

Chlorine was first used to purify water in the United States almost 100 years ago (1). Since that time, the technique has been improved upon and its use expanded so that most water systems today are disinfected (1). In 1995, approximately 64 percent of community water systems (systems that provide water to the same people year-round) specifically added chlorine as a disinfectant, and the remaining systems commonly added chloramine, according to an Environmental Protection Agency survey (1). Disinfection protects against waterborne pathogens, but chlorine reacts with organic matter in the water to form both halogenated and nonhalogenated disinfection by-products (DBPs), including trihalomethanes (THMs) (2). The Environmental Protection Agency set standards for total THM levels in community water systems after the Safe Drinking Water Act of 1974 was implemented (1). As a result, exposure to THMs is generally at levels currently deemed safe. Nevertheless, given that such exposure is pervasive throughout the United States, if DBPs at levels below the current standards were found to be associated with spontaneous abortion, it would be of public health concern.

For the past few decades, DBPs have been studied extensively for potential associations with different cancers, and, more recently, the literature has expanded to include reproductive outcomes (2–4). Only a few previous studies quantified THM exposures and examined spontaneous abortion, specifically those by Savitz et al. (5) and Waller et al. (6). The more comprehensive of these two earlier studies (6) suggested a possible association between spontaneous abortion and high consumption of total THMs (determined by a combination of high total THM exposure and high consumption of cold tap water). Of the individual THMs measured, bromodichloromethane (BDCM) had the strongest association. However, exposure assessment was limited to the quarterly average THM levels reported by water utilities serving the women's residences (6) and, in a follow-up paper, THM levels at the utility sampling site closest to a participant's home (6, 7). Although these approaches were reasonable for a preliminary investigation, even the closest site might not represent actual maternal exposure because of variability in THM levels within the system and across time. Furthermore, quarterly averages could only approximately match potential critical windows during gestation. The study by Waller et al. (6) did, however, justify a more in-depth study of THMs and spontaneous abortion.

The new study by Savitz et al. reported in this issue of the *Journal* (8) represents the state-of-the-art for a study of environmental factors and spontaneous abortion. Their attention to the exposure assessment was impressively thorough and addressed numerous deficiencies outlined in previous critical reviews (3, 9). Strengths included selecting three sites with different chlorination by-product profiles; confirming uniform distribution of DBP levels throughout the distribution system; taking weekly measurements; assessing DBP levels at multiple locations when the distribution systems were flushed with free chlorine; and developing pregnancy-specific exposure indexes, including examining exposure during specific critical windows during gestation.

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In addition, behavioral influences on exposure were evaluated by asking about and quantifying tap water consumption (including beverages made from tap water), adjusting for volatilization of DBPs during heating, inquiring about changes in tap water consumption across pregnancy, accounting for the use of water filters and bottled water, identifying water consumption during work outside the residential water system, and estimating dermal absorption and inhalation from showering and bathing.

The study's strengths were not limited to the exposure assessment. The sample size was large (2,409 pregnancies, 258 losses), with women recruited quite early in pregnancy (mean gestational age at enrollment was approximately 7 completed weeks), which is particularly desirable in a study of spontaneous abortion. In addition, the authors (8) implemented statistical methods that took into account left truncation of gestational age at enrollment.

Overall, Savitz et al. (8) did not confirm an association between DBPs and spontaneous abortion; the essentially null results were robust to a variety of parameterizations of the exposures. The authors took great pains to try to replicate the results of the study by Waller et al. (6). The confidence intervals in the two studies did overlap substantially, but the Savitz et al. results tended to be shifted closer to the null (6, 8). Notably, when Waller et al. (6) reanalyzed their data by assigning total THM based on the utility's sampling site closest to the participant's home instead of the utility-wide average, their results also tended to move slightly toward the null (7), which suggests greater consistency between the two studies than is initially apparent. Moreover, in a reanalysis of the Waller et al. data (6) using survival analysis to adjust for variable left truncation, we found hazard ratios to be closer to the null than the original odds ratios (10).

The two studies suggested that the risk of spontaneous abortion was slightly elevated for women who both were assigned the upper quartile of BDCM exposure and consumed five or more glasses per day of home tap water, compared with all others (6, 8). The relevant animal literature provides inconsistent evidence: in two studies, high doses of BDCM in water were associated with full-litter resorption in Fischer-344 rats (11, 12), but this finding was not confirmed in Sprague-Dawley rats (12). Given that the lowest dose associated with an effect was over 5,000 times the approximate human exposure from drinking water (11, 12), cross-species extrapolation is questionable. However, an in vitro study found BDCM to be associated with decreased secretion of human chorionic gonadotropin by undifferentiated human cytotrophoblast cultures; impaired production of human chorionic gonadotropin could interfere with maintenance of a pregnancy (13). Moreover, brominated compounds were associated with shorter menstrual cycles in a cohort of several hundred women (14), indicating possible alterations in ovarian function. Nevertheless, when Savitz et al. (8) categorized BDCM into quintiles, no elevated risks were observed. This finding was true for BDCM concentration alone, daily ingestion, exposure from bathing and showering, and an integrated multiroute exposure estimate.

The overall null results for THM as well as those for BDCM specifically seem unlikely to be due to confounding (8). Despite extensive study, there are few strong risk factors for spontaneous abortion besides maternal age (15). Experiencing a prior loss is also strongly associated with spontaneous abortion but not necessarily causally so (16). Savitz et al. (8) considered both of these potential confounders as well as numerous others. A few risk factors they did not account for (such as lead and nitrous oxide exposure (17, 18)) would likely have been present at low levels or not at all and, if present, were unlikely to have been associated with the exposures of interest. A larger proportion of the women from the community selected for exposures to brominated compounds were of Hispanic ethnicity (37 percent vs. 2–3 percent), had lower educational levels, and had higher parity. Although the literature does not support substantially greater risk of spontaneous abortion by education or ethnicity, Hispanic women tend to appear for prenatal care later than others (8), and adjustment for left truncation may not be adequate if there were few Hispanic women entering early. The authors' use of a random effect for community may have partially adjusted for these differences but might also have overadjusted. Thus, confounding is unlikely to account for the total THM results, but further analyses of BDCMs might be useful in addressing the possibilities of residual confounding or potential overadjustment.

Other sources of bias probably had little influence on the results. The fact that women had to contact the study staff early in pregnancy would influence selection. Could the selection be associated with factors that influence both exposure and outcome? Certainly, women who delivered livebirths would have a greater chance of entering the study than those delivering spontaneous abortions, but the authors addressed this issue by adjusting for left truncation (8). It is more difficult to imagine how self-selection would be related to the exposure, considering that assignment of DBP levels was the same for all women for a given calendar week in a given region. Information bias could be present because approximately a third of the women who experienced spontaneous abortions were interviewed after the loss. However, the DBP levels came from an independent source, and the women were asked a series of questions without any indication that DBP levels were of specific interest. Nevertheless, it would be interesting to see whether the results changed when these women were excluded.

Swimming is an intriguing possible source of misclassification. The volatile DBPs, such as THMs, may be subject to greater uptake through dermal absorption and inhalation compared with consumption (19). In fact, swimming for 1 hour has been reported to lead to a chloroform dose almost a hundred times higher than the exposure from drinking tap water (19). Thus, swimmers may in fact have the highest exposure to DBPs in the population. Unfortunately, Savitz et al. (8) were unable to include swimming in their models. A large pregnancy cohort study showed no association of swimming with risk of low birth weight (20), but spontaneous abortion occurs by mechanisms different from those affecting growth. Hence, misclassification due to omission of swimming as a source of high DBP exposures may be a source of bias in the Savitz et al. study (8) if a substantial number of women were swimmers.

Although the investigation by Savitz et al. (8) does not preclude an effect of DBPs on pregnancy, the study provides

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some confidence that exposure to THMs through most routes is not a threat to fetal viability during the first 20 weeks of gestation. Considering the public health value of controlling waterborne pathogens economically through chlorination, future studies of spontaneous abortion and THMs are probably not warranted, although studies of swimming may be useful.

At this stage, few environmental risk factors for spontaneous abortion have been identified, which could partially be due to the heterogeneous group of outcomes under the umbrella of spontaneous abortion. Perhaps it is time to reconsider the work of Kline and Stein (15), who emphasized the importance of distinguishing between losses that are chromosomally normal and those that are abnormal. However, other areas of genetic research, such as the search for genetic polymorphisms in recurrent pregnancy loss, have yielded few replicable findings in studies of sufficient size (21–25). Other methods to refine the outcome definition may hold promise: the increased use of sonograms provides the opportunity to identify the stage of fetal or embryonic development, which would allow identification of potentially more etiologically homogeneous subsets of spontaneous abortion. A focus on determining mechanisms and molecular markers may also prove fruitful for further progress in this field. Unfortunately, future studies still have to overcome demanding logistical hurdles, particularly because spontaneous abortions occur so early in pregnancy when recruitment is difficult and collection of spontaneously aborted tissue for cytogenetic analysis is not universally feasible. The stellar investigation by Savitz et al. (8) serves as a model for studies of pregnancy loss and encourages investigators to aim for the best possible study designs and the most thorough exposure determination despite the challenges.

ACKNOWLEDGMENTS

This work was supported in part by the Intramural Research Program of the National Institutes of Health, National Institute of Child Health and Human Development and by NIH grants 1 P01-ES11269, 1 R01-CA96525, and EPA STAR grants R829338 and RD83154001-1.

Dr. Penelope Howards participated in meetings during the recruitment stage of the Savitz et al. study (8) but was not involved with that publication nor was she compensated at any time with study funds. Dr. Irva Hertz-Picciotto was named in the original grant and participated in a few early meetings of the study during the design phase, but she was not involved any further.

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